Docket No. 195617US0X

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
SACHIKO MACHIDA ET AL	: EXAMINER: MOHAMED, ABDEL. A.
SERIAL NO: 09/635,429	:
FILED: AUGUST 10, 2000	: GROUP ART UNIT: 1653
FOR: ARTIFICIAL CHAPERON KIT	:

APPEAL BRIEF

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

This is an appeal from the Final Rejection of the claims dated August 13, 2002.

I. REAL PARTY IN INTEREST

The real party in interest is Director of National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries and Bio-Oriented Technology Research Advancement Institution, by virtue of the assignment recorded on May 31, 2002 at Reel/Frame 012943/0423.

II. RELATED APPEALS AND INTERFERENCES

Appellants, Appellants' legal representative, and their assignee are not aware of any appeals or interferences which will directly affect or be directly affected by or having a bearing on the Board's decision in this appeal.

III. STATUS OF THE CLAIMS

The appealed claims are Claims 31-47, the only claims in the case.

IV. STATUS OF THE AMENDMENT FILED UNDER 37 C.F.R. §1.116

(1) An Amendment and Request for Reconsideration was filed on November 13, 2002, in which Claims 9-30 were canceled and Claims 31-52 were added. In the Advisory Action dated December 2, 2002, the Examiner indicated that those amendments would be entered for the purposes of Appeal.

(2) A Request for Reconsideration was filed on January 13, 2003, in which amendments to Claims 32, 36, 40, and 47 were submitted. In the Advisory Action dated February 14, 2003, the Examiner indicated that those amendments would be entered for the purposes of Appeal. In addition, the Examiner also indicted that the amendments to Claims 32, 36, 40, and 47 would overcome the rejection under 35 U.S.C. §112, second paragraph.

(3) An Amendment is submitted herewith in order to correct a typographical error in a surfactant recited in Claim 36 and 37. Since that Amendment relates to formal matters only, entry thereof is respectfully requested.

V. THE APPEALED CLAIMS

A copy of the appealed claims is submitted in the attached Appendix I. The claims shown in the Appendix reflect entry of amendments (1)-(3) discussed in section IV above.

VII. SUMMARY OF THE INVENTION

The present invention relates to a kit for refolding denatured protein, comprising (a) a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150 and (b) a

polyoxyethylenic detergent. See the present specification at page 6, lines 2-4, and page 7, lines 14-18.

In one embodiment, the polyoxyethylenic detergent is selected from the group consisting of polyoxyethylenesorbitan ester, polyoxyethylenedodecyl ether, polyoxyethyleneheptamethylhexyl ether, polyoxyethyleneisooctylphenyl ether, polyoxyethylenenonylphenyl ether, polyoxyethylene fatty acid ester and sucrose fatty acid ester. See the present specification at the paragraph bridging pages 6 and 7.

In another embodiment, the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50. See the present specification at page 7, line 18.

In another embodiment, the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150. See the present specification at page 7, line 18.

The present invention also relates to a kit for refolding denatured protein, comprising (a) a cyclic saccharide cycloamylose having a polymerization degree of from 25 to 150 and (b) an ionic detergent. See the present specification at page 5, lines 17-21, and page 7, line 18.

In one embodiment, the ionic detergent is selected from the group consisting of cetyltrimethylammonium bromide, sodium dodecyl sulfate, sodium deoxycholate, 3-[3-colamidopropyl)dimethylamino]-1-propane sulfonic acid, hexadecyltrimethylammonium bromide and myristylsulfobetaine. See the present specification at page 7, lines 7-13.

In another embodiment, the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50. See the present specification at page 7, line 18.

In another embodiment, the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150. See the present specification at page 7, line 18.

The present invention also relates to a method of refolding a denatured protein, comprising:

contacting a polyoxyethylenic detergent with a denatured protein, followed by

contacting the protein with a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150, to produce a folded protein. See the present specification at page 6, lines 2-4; page 7, lines 14-18, and page 11, lines 10-13.

In one embodiment, the polyoxyethylenic detergent is selected from the group consisting of polyoxyethylenesorbitan ester, polyoxyethylenedodecyl ether, polyoxyetheyleneheptamethylhexyl ether, polyoxyethyleneisooctylphenyl ether, polyoxyethylenenonylphenyl ether, polyoxyethylene fatty acid ester and sucrose fatty acid ester. See the present specification at the paragraph bridging pages 6 and 7.

In another embodiment, the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50. See the present specification at page 7, line 18.

In another embodiment, the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150. See the present specification at page 7, line 18.

In another embodiment, the folded protein has an α-helical structure. See the present specification at page 11, line 16.

In another embodiment, the folded protein has an β-sheet structure. See the present specification at page 8, line 20.

In another embodiment, the refolded protein has an intramolecular S-S bond. See the present specification at page 8, line 21.

The present invention also relates to a method of refolding a denatured protein, comprising:

contacting an ionic detergent with a denatured protein, followed by

contacting the protein with a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150, to produce a folded protein. See the present specification at page 5, lines 17-21; page 7, line 18; and page 11, lines 10-13.

In one embodiment, the ionic detergent is selected from the group consisting of cetyltrimethylammonium bromide, sodium dodecyl sulfate, sodium deoxycholate, 3-[3-colamidopropyl)dimethylamino]-1-propane sulfonic acid, hexadecyltrimethylammonium bromide and myristylsulfobetaine. See the present specification at page 7, lines 7-13.

In another embodiment, the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50. See the present specification at page 7, line 18.

In another embodiment, the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150. See the present specification at page 7, line 18.

In another embodiment, the folded protein has an α-helical structure. See the present specification at page 11, line 16.

In another embodiment, the folded protein has an β-sheet structure. See the present specification at page 8, line 20.

In another embodiment, the refolded protein has an intramolecular S-S bond. See the present specification at page 8, line 21.

VIII. THE ISSUE OF THIS APPEAL

The sole issue in this appeal is whether Claims 31-52 are unpatentable under 35 U.S.C. §103(a) as being obvious over Daugherty et al. (The Journal of Biological Chemistry, Vol. 273, No. 51, pp. 33961-33971, December 18, 1998) taken with Takaha et al. (The Journal of Biological Chemistry, Vol. 271, No. 6, pp. 2902-2908, February 9, 1996).

IX. GROUPING OF THE CLAIMS

The claims do not stand or fall together. The reasons for them not standing or falling together with the other claims will be pointed out and discussed below.

X. ARGUMENT IN TRAVERSAL OF THE REJECTION

As discussed in the present specification at page 5, first full paragraph, the present invention is based on the discovery that the larger cyclic saccharide cycloamylose recited in the pending claims, i.e., having a degree of polymerization of 25 to 150, in combination with a polyoxyethylenic detergent (independent Claims 31 and 39) or an ionic detergent (independent Claims 35 and 46), overcomes problems associated with β-cyclodextrin. As described in the specification at the bottom of page 3, the β-cyclodextrin used by Daugherty et al. has problems associated with stability, and is not completely satisfactory.

There is no *prima facie* case of obviousness with respect to Daugherty et al. in view of Takaha et al.

Claim 31

Daugherty et al. describe protein refolding using β-cyclodextrin. See the Table at page 33963 of the reference. In fact, β-cyclodextrin is the only cyclic saccharide described therein. The β-cyclodextrin used in Daugherty et al. has a polymerization degree of 7 (See Machida et al. FEBS Letters, 486, (2000), pp. 131-135, of record, at page 131, second column, second full paragraph, lines 1-3). Therefore, Daugherty et al. fail to describe a cyclic saccharide cycloamylose having a polymerization degree of from 25-150 in combination with the claimed detergents as a protein refolding agent.

Takaha et al. describe producing cycloamylose using potato D-enzyme (see the Abstract). The reference is directed to the physical characterization of the cycloamylose product of the potato D-enzyme described therein. Takaha et al. fail to describe using the cycloamylose prepared therein as a protein refolding agent This reference fails to describe protein refolding with the cycloamylose. In the paragraph bridging pages 2907 and 2908 of the reference, Takaha et al. describe several properties of the cycloamylose, and speculate that:

> there is great potential for the exploitation of cycloamylose in chemical, pharmaceutical, and food industries to safely achieve the solubilization, increased stability, sequestration, or altered reactivity of molecules with which it can form inclusion complexes.

Takaha et al. clearly fail to even mention using the cycloamylose as a protein refolding agent.

These references, taken in combination, fail to suggest the claimed invention.

There is simply no suggestion in these references to substitute the cycloamylose described in Takaha et al. for the β-cyclodextrin described in Daugherty et al. Daugherty et al. do not describe any shortcomings of β-cyclodextrin which would motivate one to use a different cyclodextrin. Takaha et al. reports the physical characterization of the cycloamylose described therein. In the Official Action dated August 13, 2002, at page 5, the Examiner stated that Takaha et al. "discusses...the potential applications for cycloamylose." Significant by its absence is any mention of using the cycloamylose as a protein refolding agent. Therefore, these references fail to suggest the claimed kits and methods.

In the Advisory Action, the Examiner has taken the position that a side-by-side comparison must be performed between the claimed cyclic saccharide cycloamylose and β-cyclodextrin in combination with the recited detergents. However, as discussed in the

previous response, the present specification does present such data. That is to say, Table 1 shows the experimental result of refolding a protein, where Tween 40 was used as a detergent in combination with various cyclic saccharides. In comparing the refolding effect on denatured by each combination, cyclic saccharide cycloamylose CA(S) and CA(L) having a polymerization degree of 25 to 150 recovered 140% and 120% of activity, respectively. β-cyclodextrin of polymerization degree of 7 recovered 120% of activity as well, but this substance has a defect as described above. The result of γ-cyclodextrin having a degree of polymerization 8 was 8% of the recovery and no refolding effect was observed. A similar experiment was carried out with a cyclic saccharide having a polymerization degree of 10 to 14, resulting in a recovery of only 4% of activity. Therefore, all the cyclic saccharides having various polymerization degrees do not always show a refolding effect. It is not expected from the cited references that the cyclic saccharide cycloamylose of the present invention, which has a much higher polymerization degree than the β-cyclodextrin of polymerization degree of 7, yields a far superior refolding effect.

In the Example 1 of the present application, citrate synthase (CS) is first denatured with guanidine hydrochloride and then refolded with a variety of artificial chaperones. See page 10 and page 13 of the specification. The results are presented in Table 1 at page 18 of the present specification.

The results of this experiment are set forth at page 19, first two paragraphs, of the specification which read as follows:

> Moreover, as to the change with the passage of time of the enzymatic activity, as apparent from Figs. 1 and 2, it has become clear that, in case of the artificial chaperon using CA(S) and CA(L) as the cyclic saccharide, the enzyme was refolded into the active form within as short as 2 hours after the addition of cycloamylose. That is, this shows that the artificial chaperon of the present invention has the ability of refolding

the denatured protein in an unfolded state correctly within a short time.

On the other hand, in case of β-CD, only from about 30 to 40% of the enzymatic activity was recovered 2 hours after the addition of the β-CD, and it took more than overnight to recover 100% of the enzymatic activity.

Therefore, it has become clear that cycloamylose is more preferable agent used as the artificial chaperon of the present invention.

Thus, the present specification provides the very side-by-side comparative data that the Examiner is looking for. As described at page 17 of the specification, Figure 1 of the present application presents the time course of the recovery of enzymatic activity using different cyclic saccharides and Tween 40, and Figure 2 presents similar data for Tween 60. As stated in the passage from the specification described above, using the claimed re-folding agent the enzyme was refolded into the native form within as short a time period as 2 hours. In contrast, with β-cyclodextrin, only about 30 to 40% of the enzymatic activity was recovered in 2 hours, and it took more than an overnight incubation to recover 100% of the enzymatic activity.

The cited references fail to suggest these striking results.

Daugherty et al. describe protein refolding using β-cyclodextrin. This reference fails to describe the cyclic saccharide cycloamylose having a polymerization degree of from 25-150 in combination with the specified detergents as claimed.

Takaha et al. describe producing cycloamylose with potato D-enzyme (see the Abstract). Nothing in this reference suggests using the a cyclic saccharide cycloamylose having a polymerization degree of from 25-150 in combination with the specified detergents for refolding proteins. Rather, this reference describes the physical characterization of the

cycloamylose product of the potato D-enzyme described therein. Thus, this reference contains no teaching or description of protein refolding whatsoever.

One with Daugherty et al. and Takaha et al. in hand would not have predicted that a cyclic saccharide cycloamylose having a polymerization degree of from 25-150 would be dramatically more effective as compared to β-cyclodextrin for refolding proteins as demonstrated by the data presented in the present specification. Daugherty et al. describe protein refolding using β-cyclodextrin and is silent with respect to a cyclic saccharide cycloamylose having a polymerization degree of from 25-150. Takaha et al. describe a a cyclic saccharide cycloamylose within the scope of the claims, but is silent with respect to refolding proteins. Given the teachings of the references, one would simply not be led to expect the striking results set forth in the present specification.

In the Advisory Action dated February 14, 2003, the Examiner criticized the experimental data discussed above on the grounds that "there is nothing in the claims to tie decreased refolding time and stability of the protein to the components of the kit and methods of refolding denatured protein." In response, Appellants note that the decreased refolding time and stability of the protein are inherent properties of the claimed kit and the use of the components of the kit in the claimed method. The purpose of the claims is to define the invention. The results obtained with the kit, i.e., decreased refolding time and stability of the protein, are described in detail in the specification of the present application, and the patentability of the pending claims does not require explicit recitation of those properties in the claims.

Based on the foregoing, the combination of Daugherty et al. and Takaha et al. fail to suggest the claimed kit. Therefore, Claim 31 is not obvious over those references.

Claim 32

Claim 32 depends from Claim 31 and recites that the polyoxyethylenic detergent is selected from the group consisting of polyoxyethylenesorbitan ester, polyoxyethylenedodecyl ether, polyoxyethyleneheptamethylhexyl ether, polyoxyethyleneisooctylphenyl ether, polyoxyethylenenonylphenyl ether, polyoxyethylene fatty acid ester and sucrose fatty acid ester. The combination of Daugherty et al. and Takaha et al. fail to suggest the kit specified in Claim 31, as discussed above. Those references certainly fail to suggest such a kit where the polyoxyethylenic detergent is selected from the group recited in Claim 32. Therefore, Claim 32 is not obvious over Daugherty et al. and Takaha et al.

Claim 33

Claim 33 depends from Claim 31 and recites that the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50. The combination of Daugherty et al. and Takaha et al. fail to suggest the kit specified in Claim 31, as discussed above. Those references certainly fail to suggest such a kit where the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50. Therefore, Claim 33 is not obvious over Daugherty et al. and Takaha et al.

Claim 34

Claim 34 depends from Claim 31 and specifies that the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150. The combination of Daugherty et al. and Takaha et al. fail to suggest the kit specified in Claim 31, as discussed above. Those references certainly fail to suggest such a kit where the cyclic saccharide cycloamylose

has a polymerization degree of from 40 to 150. Therefore, Claim 34 is not obvious over Daugherty et al. and Takaha et al.

Claim 35

Claim 35 is independent and recites a kit for refolding denatured protein, comprising (a) a cyclic saccharide cycloamylose having a polymerization degree of from 25 to 150 and (b) an ionic detergent. The combination of Daugherty et al. and Takaha et al. fail to suggest the kit specified in Claim 31, as discussed above. Claim 35 differs from Claim 31 by reciting an ionic detergent instead of a a polyoxyethylenic detergent. Claim 35 is not obvious over Daugherty et al. and Takaha et al. for the same reasons as Claim 31.

Claim 36

Claim 36 depends from Claim 35 and specifies that the ionic detergent is selected from the group consisting of cetyltrimethylammonium bromide, sodium dodecyl sulfate, sodium deoxycholate, 3-[3-colamidopropyl)dimethylamino]-1-propane sulfonic acid, hexadecyltrimethylammonium bromide and myristylsulfobetaine.. The combination of Daugherty et al. and Takaha et al. fail to suggest the kit specified in Claim 35, as discussed above. Those references certainly fail to suggest such a kit where the detergent is selected from the group recited in Claim 36. Therefore, Claim 36 is not obvious over Daugherty et al. and Takaha et al.

Claim 37

Claim 37 depends from Claim 35 and specifies that the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50. The combination of Daugherty

et al. and Takaha et al. fail to suggest the kit specified in Claim 35, as discussed above. Those references certainly fail to suggest such a kit where the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50. Therefore, Claim 37 is not obvious over Daugherty et al. and Takaha et al.

Claim 38

Claim 38 depends from Claim 35 and recites that the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150. The combination of Daugherty et al. and Takaha et al. fail to suggest the kit specified in Claim 35, as discussed above. Those references certainly fail to suggest such a kit where the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150. Therefore, Claim 38 is not obvious over Daugherty et al. and Takaha et al.

Claim 39

Claim 39 is independent and recites a method of refolding a denatured protein, comprising:

contacting a polyoxyethylenic detergent with a denatured protein, followed by

contacting the protein with a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150, to produce a folded protein.

Thus, Claim 39 is directed to a method of refolding a denatured protein using the components of the kit recited in Claim 31. Accordingly, Claim 39 is not obvious over Daugherty et al. and Takaha et al. for the same reasons as Claim 31.

Claim 40

Claim 40 depends from Claim 39 and specifies that the polyoxyethylenic detergent is selected from the group consisting of polyoxyethylenesorbitan ester, polyoxyethylenedodecyl ether, polyoxyethyleneheptamethylhexyl ether, polyoxyethyleneisooctylphenyl ether, polyoxyethylenenonylphenyl ether, polyoxyethylene fatty acid ester and sucrose fatty acid ester. The combination of Daugherty et al. and Takaha et al. fail to suggest the method specified in Claim 39, as discussed above. Those references certainly fail to suggest such a method where the polyoxyethylenic detergent is selected from the group recited in Claim 40. Therefore, Claim 40 is not obvious over Daugherty et al. and Takaha et al.

Claim 41

Claim 41 depends from Claim 39 and recites that the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50. The combination of Daugherty et al. and Takaha et al. fail to suggest the method specified in Claim 39, as discussed above. Those references certainly fail to suggest such a method where the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50. Therefore, Claim 41 is not obvious over Daugherty et al. and Takaha et al.

Claim 42

Claim 42 depends from Claim 39 and specifies that the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150. The combination of Daugherty et al. and Takaha et al. fail to suggest the method specified in Claim 39, as discussed above. Those references certainly fail to suggest such a method where the cyclic saccharide

cycloamylose has a polymerization degree of from 40 to 150. Therefore, Claim 42 is not obvious over Daugherty et al. and Takaha et al.

Claim 43

Claim 43 depends from Claim 39 and recites that the folded protein has an α-helical structure. The combination of Daugherty et al. and Takaha et al. fail to suggest the method specified in Claim 39, as discussed above. Those references certainly fail to suggest such a method where the folded protein has an α-helical structure. Therefore, Claim 43 is not obvious over Daugherty et al. and Takaha et al.

Claim 44

Claim 44 depends from Claim 39 and specifies that the folded protein has an β-sheet structure. The combination of Daugherty et al. and Takaha et al. fail to suggest the method specified in Claim 39, as discussed above. Those references certainly fail to suggest such a method where the folded protein has a β-sheet structure. Therefore, Claim 44 is not obvious over Daugherty et al. and Takaha et al.

Claim 45

Claim 45 depends from Claim 39 and recites that the refolded protein has an intramolecular S-S bond. The combination of Daugherty et al. and Takaha et al. fail to suggest the method specified in Claim 39, as discussed above. Those references certainly fail to suggest such a method where the refolded protein has an intramolecular S-S bond. Therefore, Claim 45 is not obvious over Daugherty et al. and Takaha et al.

Claim 46

Claim 46 is independent and recites a method of refolding a denatured protein, comprising:

contacting an ionic detergent with a denatured protein, followed by

contacting the protein with a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150, to produce a folded protein.

Thus, Claim 46 is directed to a method of refolding a denatured protein using the components of the kit recited in Claim 35. Accordingly, Claim 46 is not obvious over Daugherty et al. and Takaha et al. for the same reasons as Claim 35.

Claim 47

Claim 47 depends from Claim 46 and specifies that the ionic detergent is selected from the group consisting of cetyltrimethylammonium bromide, sodium dodecyl sulfate, sodium deoxycholate, 3-[3-colamidopropyl)dimethylamino]-1-propane sulfonic acid, hexadecyltrimethylammonium bromide and myristylsulfobetaine. The combination of Daugherty et al. and Takaha et al. fail to suggest the kit specified in Claim 46, as discussed above. Those references certainly fail to suggest such a method where the detergent is selected from the group recited in Claim 47. Therefore, Claim 47 is not obvious over Daugherty et al. and Takaha et al.

Claim 48

Claim 48 depends from Claim 46 and specifies that the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50. The combination of Daugherty et al. and Takaha et al. fail to suggest the method specified in Claim 46, as discussed above.

Those references certainly fail to suggest such a method where the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50. Therefore, Claim 48 is not obvious over Daugherty et al. and Takaha et al.

Claim 49

Claim 49 depends from Claim 46 and specifies that the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150. The combination of Daugherty et al. and Takaha et al. fail to suggest the method specified in Claim 46, as discussed above. Those references certainly fail to suggest such a method where the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150. Therefore, Claim 49 is not obvious over Daugherty et al. and Takaha et al.

Claim 50

Claim 50 depends from Claim 46 and specifies that the folded protein has an α-helical structure. The combination of Daugherty et al. and Takaha et al. fail to suggest the method specified in Claim 50, as discussed above. Those references certainly fail to suggest such a method where the folded protein has an α-helical structure. Therefore, Claim 50 is not obvious over Daugherty et al. and Takaha et al.

Claim 51

Claim 51 depends from Claim 46 and specifies that the folded protein has an β-sheet structure. The combination of Daugherty et al. and Takaha et al. fail to suggest the method specified in Claim 46, as discussed above. Those references certainly fail to suggest such a

method where the folded protein has a β-sheet structure. Therefore, Claim 51 is not obvious over Daugherty et al. and Takaha et al.

Claim 52

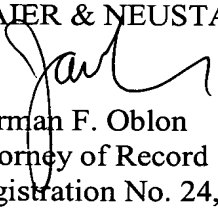
Claim 52 depends from Claim 46 and specifies that the refolded protein has an intramolecular S-S bond. The combination of Daugherty et al. and Takaha et al. fail to suggest the method specified in Claim 46, as discussed above. Those references certainly fail to suggest such a method where the refolded protein has an intramolecular S-S bond. Therefore, Claim 52 is not obvious over Daugherty et al. and Takaha et al.

XI. RELIEF REQUESTED

Reversal of the Examiner's rejection of the appealed claims under 35 U.S.C. §103(a) is requested.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Norman F. Oblon
Attorney of Record
Registration No. 24,618

James J. Kelly, Ph.D.
Registration No. 41,504

Fourth Floor
1755 Jefferson Davis Highway
Arlington, Virginia 22202
(703) 413-3000
Fax #: (703) 413-2220
NFO/JK

I:\atty\JK\2003\01-03\195617US.AB.wpd

APPENDIX I

The appealed claims read as follows:

31. A kit for refolding denatured protein, comprising (a) a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150 and (b) a polyoxyethylenic detergent.

32. The kit of Claim 31, wherein the polyoxyethylenic detergent is selected from the group consisting of polyoxyethylenesorbitan ester, polyoxyethylenedodecyl ether, polyoxyethyleneheptamethylhexyl ether, polyoxyethyleneisooctylphenyl ether, polyoxyethylenenonylphenyl ether, polyoxyethylene fatty acid ester and sucrose fatty acid ester.

33. The kit of Claim 31, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50.

34. The kit of Claim 31, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150.

35. A kit for refolding denatured protein, comprising (a) a cyclic saccharide cycloamylose having a polymerization degree of from 25 to 150 and (b) an ionic detergent.

36. The kit of Claim 35, wherein the ionic detergent is selected from the group consisting of cetyltrimethylammonium bromide, sodium dodecyl sulfate, sodium deoxycholate, 3-[3-colamidopropyl)dimethylamino]-1-propane sulfonic acid, hexadecyltrimethylammonium bromide and myristylsulfobetaine.

37. The kit of Claim 35, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50.

38. The kit of Claim 35, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150.

39. A method of refolding a denatured protein, comprising:

contacting a polyoxyethylenic detergent with a denatured protein, followed by

contacting the protein with a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150, to produce a folded protein.

40. The method of Claim 39, wherein the polyoxyethylenic detergent is selected from the group consisting of polyoxyethylenesorbitan ester, polyoxyethylenedodecyl ether, polyoxyethyleneheptamethylhexyl ether, polyoxyethyleneisooctylphenyl ether, polyoxyethylenenonylphenyl ether, polyoxyethylene fatty acid ester and sucrose fatty acid ester.

41. The method of Claim 39, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50.

42. The method of Claim 39, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150.

43. The method of Claim 39, wherein the folded protein has an α-helical structure.

44. The method of Claim 39, wherein the folded protein has an β-sheet structure.

45. The method of Claim 39, wherein the refolded protein has an intramolecular S-S bond.

46. A method of refolding a denatured protein, comprising:

contacting an ionic detergent with a denatured protein, followed by

contacting the protein with a cyclic saccharide cycloamylose having a degree of polymerization of 25 to 150, to produce a folded protein.

47. The method of Claim 46, wherein the ionic detergent is selected from the group consisting of cetyltrimethylammonium bromide, sodium dodecyl sulfate, sodium deoxycholate, 3-[3-colamidopropyl)dimethylamino]-1-propane sulfonic acid, hexadecyltrimethylammonium bromide and myristylsulfobetaine.

48. The method of Claim 46, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 25 to 50.

49. The method of Claim 46, wherein the cyclic saccharide cycloamylose has a polymerization degree of from 40 to 150.

50. The method of Claim 46, wherein the folded protein has an α-helical structure.

51. The method of Claim 46, wherein the folded protein has an β-sheet structure.

52. The method of Claim 46, wherein the refolded protein has an intramolecular S-S bond.